

## OPTIMISATION OF pH AND NUTRIENT REQUIREMENTS FOR THE BIOLOGICAL DEGRADATION OF PHARMACEUTICAL WASTEWATER

\*S. Yousaf<sup>1</sup> and Z.M. Khalid<sup>2</sup>

<sup>1</sup>PPD, NIAB, P. O. Box # 128, Faisalabad, Pakistan

<sup>2</sup>EBD, NIBGE, P. O. Box # 577, Faisalabad, Pakistan

---

### ABSTRACT

The pH conditions were optimised for the biodegradation of pharmaceutical wastewater. Wastewater treatment in the presence and absence of added nutrients of nitrogen and phosphorous was also checked at various pH levels. Different ratios of nitrogen (N) and phosphorous (P) were employed to optimise nutrient requirement. Almost all treatments resulted in 90 to 98% decrease in COD. The results of decrease of COD with the passage of time were almost similar for both nutrient supplemented or non-supplemented samples. Thus there is no need of adding nitrogen and phosphorous as nutrient supplement. The influence of acidic, basic, and neutral pH conditions on biodegradation was also checked; acidic pH, which is the actual pH of the samples, was proved to be the best than at basic and neutral pH conditions. At basic pH solubilization of COD occurs.

**Key words:** Biodegradation, pharmaceutical waste, wastewater treatment, pH optimisation, nutrient optimisation

---

### INTRODUCTION

The use of polluted waters has been reported to have adverse effect on the livestock and vegetation (Bokhari and Malik, 1997) and soil and vegetation (Khan and Akif, 1994). A previous study (Yousaf and Khalid, 2004) investigated the aerobic degradation/detoxification of wastewater from antibiotic producing pharmaceutical industry.

While manufacturing antibiotics like ampicillin/amoxycillin, chemicals like methylene chloride (MC), tri-ethyl amine (TEA), pyridine and CIMC chlorides [3-(2- Chlorophenyl)-5-methyl isoxazole carbonyl chloride] are used, which are very toxic. Some of them like pyridine and MC are confirmed carcinogens. In view of potential hazards of these toxicants to human health, control for their discharge to environment is necessary. Due to high concentration of organics this wastewater is separated into two different layers, upper organic and lower aqueous layer. In this respect microbiological means for the treatment of pharmaceutical wastewater were tried and such microorganisms have been isolated which can degrade this effluent. Degradation is measured in terms of decrease in Chemical Oxygen Demand (COD).

Penaud *et al.* (1997) have reported the effect of pH on the percentage of COD solubilization and inferred that COD solubilization increased progressively when the pH rose from 5 to 8 and increased sharply at pH greater than 8. The purpose of this study was to check the effect of added nutrients on degradation and to optimise the operational conditions of the pH for COD removal.

### MATERIALS AND METHODS

#### *Sampling*

Six effluent samples were collected from different sections of a pharmaceutical industry namely, isopropyl alcohol residue, methylene chloride residue, ampicillin effluent, amoxycillin effluent, washing effluent and septic waste. These samples were stored at 4°C in glass bottles; pH of each sample was recorded in the laboratory, using HANNA 8520 digital pH meter.

#### *Microorganisms*

Already isolated (Yousaf and Khalid, 2004) consortium of bacteria (*Pseudomonas* and *Bacillus*) was employed as inoculum. These bacterial strains capable of growing on effluent samples individually and on their mixture were isolated from sewage sludge using enrichment techniques like shake flask and air lift percolators.

#### *Nutrients*

In the reaction mixture nitrogen (N) and phosphorous (P) were added as primary nutrients. Source of nitrogen and phosphorous were (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and KH<sub>2</sub>PO<sub>4</sub>/K<sub>2</sub>HPO<sub>4</sub> respectively.

---

\*Corresponding author: S. Yousaf, PPD, NIAB, P. O. Box # 128, Faisalabad, Pakistan

### Analytical methods

The amount of organic matter in the wastewater was measured as COD, with the method reported by Jirka and Corter (1975). The amount of  $\text{NH}_4$ -nitrogen was measured by Kjeldahal method (Bremner and Mulvaney, 1982) and  $\text{PO}_4$ -phosphorous were measured with the Olsen and Somer (1982) test.

### Experimental methods

**Optimisation of Nutrients:** All effluent samples were mixed equally, diluted with water (distilled water) in a ratio of 1:40, supplemented with mineral media containing nitrogen (N) and phosphorous (P) as primary nutrients in various ratios and then inoculated with already isolated strains.  $(\text{NH}_4)_2\text{SO}_4$ ,  $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$  were used as source of nitrogen (N) & phosphorous (P), respectively. Nineteen (19) different ratios of nitrogen (N) & phosphorous (P), (N: P; 1:2, 1:1, 2:1, 1:3, 3:1, 1:4, 4:1, 1:5, 5:1, 2:3, 3:2, 3:4, 4:3, 2:5, 5:2, 3:5, 5:3, 4:5, 5:4) were used to optimise nutrient requirement. Shake flask experiment was performed to optimise the nutrients requirement for the reduction of COD. The culture medium contained, 50 ml effluent sample, 50 ml water and particular amount of N and P from 1%  $(\text{NH}_4)_2\text{SO}_4$  and 2 %  $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$  in 250 ml Erlenmeyer flasks. The medium was inoculated and incubated at  $25^\circ\text{C}$  in an orbital shaker at 100 rev./min. and COD removal was checked with the passage of time. Sampling was done immediately after inoculation and than after 3, 7, 11 and 21 days.

Table 1. Results of decrease in Chemical Oxygen Demand (COD) values with the passage of time in shake flask experiment, for effluent mixture and water in a ratio of 1:40, using different ratios of added nutrients of nitrogen (N) and phosphorous (P).

N:P ratios	Percent decrease in COD (mg/l) with the passage of time (days)					
	0	5	8	11	15	21
1:2	0	82.3	86.3	92.7	93.2	93.4
1:1	0	67.7	78.4	87.9	90.3	92.0
2:1	0	89.5	92.6	95.3	96.4	96.9
1:3	0	68.3	72.5	81.1	83.7	86.9
3:1	0	89.4	93.6	96.8	95.2	96.5
1:4	0	81.9	92.2	95.5	95.7	96.0
4:1	0	47.3	81.5	85.3	93.2	93.2
1:5	0	72.8	87.8	92.3	93.1	93.3
5:1	0	90.0	95.5	97.1	97.4	98.0
2:3	0	13.7	65.9	83.8	76.9	84.5
3:2	0	87.3	85.7	93.5	86.7	92.9
3:4	0	48.1	78.2	87.0	87.3	87.5
4:3	0	68.3	83.1	89.6	90.3	93.3
2:5	0	83.4	88.5	94.4	93.1	94.2
5:2	0	90.0	92.0	98.4	94.6	95.8
3:5	0	87.6	82.1	88.0	86.5	91.3
5:3	0	96.1	95.6	96.2	96.8	97.0
4:5	0	85.1	92.1	96.8	96.3	96.4
5:4	0	86.8	85.5	91.2	91.5	91.7

**Optimisation of pH:** After checking the initial pH of the effluent samples, the effect of acidic and basic pH conditions on the reduction of COD values was checked by adding N and P in ratios of 5:2 and 2:5 (already optimised), using mixture of the effluent samples. The effect of different pH conditions, acidic, basic and neutral was also checked on higher substrate concentration (1:4) using nitrogen (N) and phosphorous (P) ratios of 5:2 and 2:5. In continuation with this experiment the biodegradation of same samples was studied without adding nitrogen and phosphorous as primary nutrients.

## RESULTS AND DISCUSSION

For all of the six effluent samples collected from a pharmaceutical industry pH values of 3.83, 3.77, 3.44, 8.52, 4.84 and 8.20 were recorded for isopropyl alcohol residue, methylene chloride residue, ampicillin effluent, washing effluent, amoxycillin effluent, and septic waste respectively. All effluent samples when mixed equally, resulted in 40% non-aqueous (organic) top layer and 60% aqueous lower layer. General appearance of the top layer was brown coloured organic liquid and lower layer was of yellow colour. pH values of upper and lower layers were found to be 3.3 and 3.2 respectively. Although washing effluent and septic waste effluent had pH values of 8.52 and 8.20 but they had no buffering capacity and thus the mixture was found to be of pH value  $3 \pm 0.3$ .

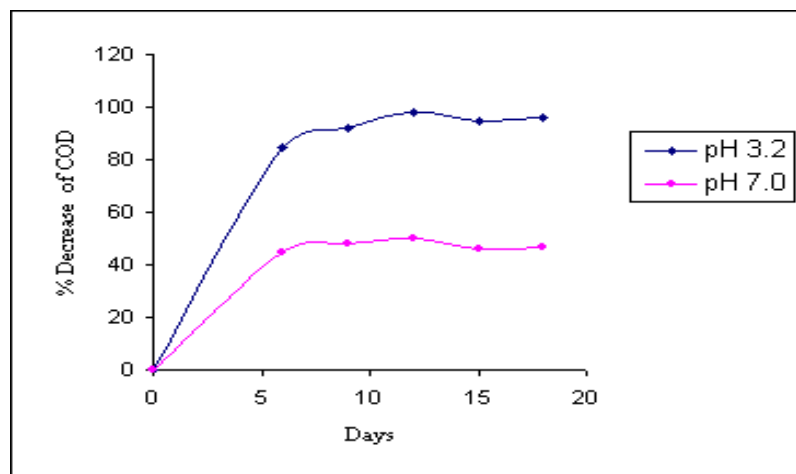


Fig. 1. Effect of pH (acidic and neutral) on decrease of COD using substrate concentration (1: 40) and N: P (5: 2) at 32°C

For optimising nutrient (N and P) requirement for biodegradation of equally mixed effluent at actual acidic pH value of  $3 \pm 0.3$ , different ratios of nitrogen and phosphorous were tried in shake flask (Table 1). Almost all treatments resulted in 90 to 98% decrease in COD. During this experimentation amount of N and P were routinely checked, with the passage of time. Estimation of nitrogen and phosphorous values resulted in an increase in nitrogen and no change was observed on phosphorous, as degradation proceeded. This increase of nitrogen might be due to the presence of some nitrogen fixing bacterial spp. And no change in the values of total phosphorous indicated that required nutrients were present in the effluents in sufficient amount to support the growth of bacteria. Thus there is no need of adding nitrogen and phosphorous as nutrient supplement.

From the checked N: P ratios (Table 1); ratios of 5:2 and 2:5 were selected for further studies. Using upper and lower layers with water in a ration of 1: 4 biodegradation studies were carried out at actual acidic pH and also at basic pH of 11, which was adjusted by using NaOH. Solubilization of upper and lower layers was observed with the increase of pH, this result is in consistent with those reported by Penaud *et al.* Fig.1 represents the effect of pH on decrease of COD in N: P ratio 5:2 at 32°C. Using N and P in ratio of 5:2, 90% decrease in COD was observed after 6 days at acidic pH, but at basic pH this decrease in COD was only 45.8%. Similarly when N and P were used in a ratio of 2:5, using same concentration of samples, again after 6 days 95% decrease in COD was observed at acidic pH and at basic pH of 11 the extent of decrease in COD was less. This can be explained on the basis of solubilization of COD with the increase of pH as reported by Penaud *et al.* Fig. 2 represents the effect of pH on COD reduction using N: P ratio of 2:5 at 32°C.

Influence of pH on biodegradation was also studied using higher concentrations of mixture of upper and lower layers with water (1:4) at 32°C. Effect of added nutrients upon biodegradation was also checked. When N and P were used in a ratio of 5:2, again acidic pH of 3 was found to be the best for the degradation of this type of effluent by isolated consortia. The pH value of 7 gave more favourable results than at pH value of 11, but not better than at pH 3.0. So pH 3, which is the actual pH, is the usual value of pH for this biodegradation. Fig.3 represents the results of COD reduction at different pH conditions using N: P ratio 5:2. For higher substrate concentration (1:4) using N: P ratio of 5:2, again acidic pH of 3 was found to be more suitable than basic and neutral pH conditions. Fig. 4 represents the effect of different pH conditions on COD reduction using N: P ratio 5:2. Biodegradation was also checked in the absence of added nutrients at three different pH levels of acidic, basic and neutral and there absence resulted no pronounced effect on degradation. So it is concluded that isolated and developed consortia do not require N & P as supplement and can utilize concerned wastewater as sole source of energy. Fig.5 represents the effect of different pH conditions on COD reduction in the absence of added nutrients (N, P) in this case again the acidic pH was proved best than basic and neutral pH conditions.

Time course studies for this experiment, regarding higher substrate concentration (1:4) demonstrated only 65% decrease in COD after 19 days as compared to 95% decrease in COD after 6 days using substrate concentration of 1:40, probably because of higher concentrations of organics in the sample.

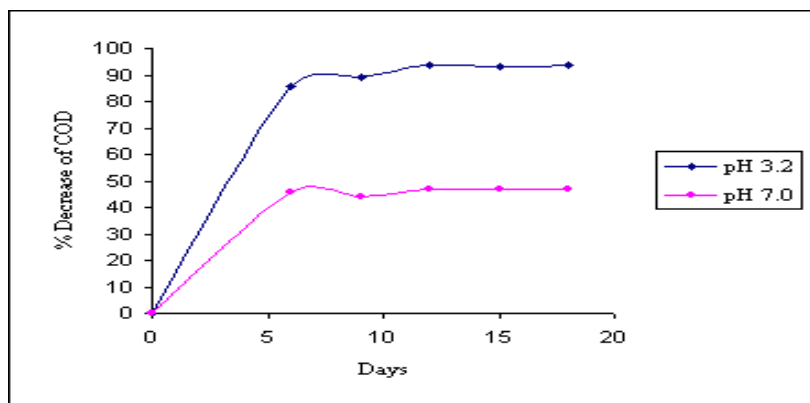


Fig. 2. Effect of pH (acidic and neutral) on decrease of COD using substrate concentration (1: 40) and N: P (2: 5) at 32°C.

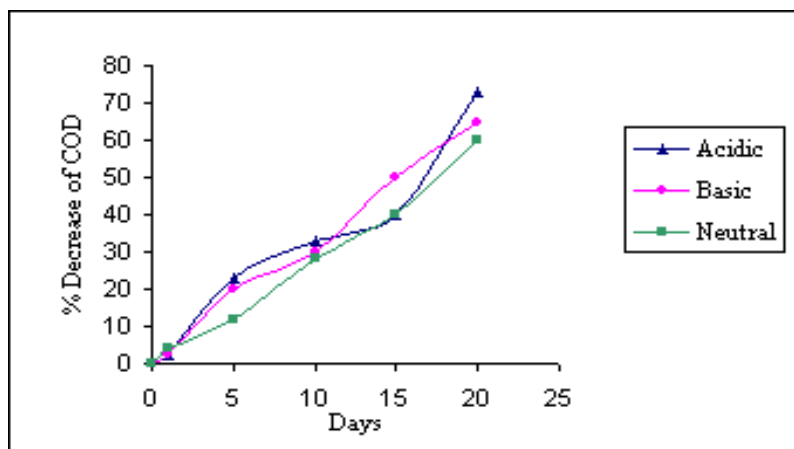


Fig. 3. Effect of pH on reduction of COD using substrate concentration (1: 4) and N: P (5: 2) at 30°C.

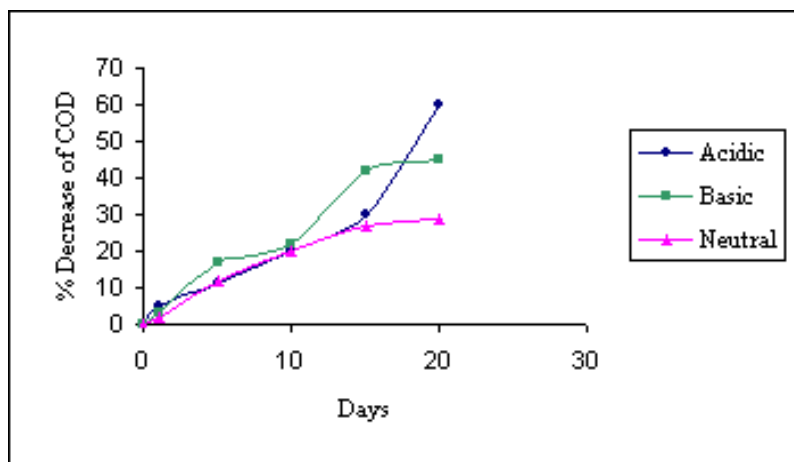


Fig. 4. Effect of pH on reduction of COD using substrate concentration (1: 4) and N: P (2: 5) at 30°C.

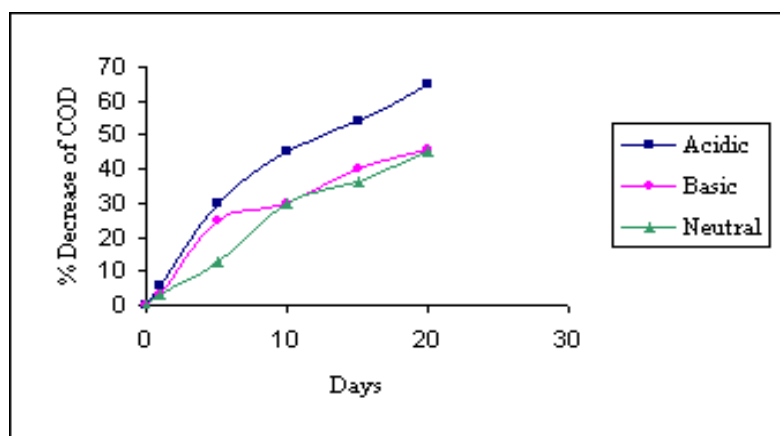


Fig. 5. Effect of pH on reduction of COD using substrate concentration (1: 4) in the absence of added nutrients.

## CONCLUSIONS

This study indicates that because of higher concentrations of organics and their toxic nature, the optimisation of operational conditions of pH and nutrients for the biodegradation of this pharmaceutical wastewater is necessary for its treatment. Acidic pH of 3, which is also the actual pH of the effluent sample, is found to be more suitable as compared to neutral and basic pH values. With the increase of pH, solubilization of COD occurs and it becomes less available to microorganisms. The application of increasing substrate concentration leads to decreasing COD reduction. No additional influence of added nutrients was observed on biodegradation.

## REFERENCES

- Bremner, J. M. and C.S. Mulvaney (1982). Nitrogen total *In: Methods of soil analysis part 2. Chemical and Microbiological properties.* (Page A1. ed). Am.Soc. Agron. Inc. Madison, Wis. pp: 599-6241.
- Bokhari, M. H. and S. A. Malik (1997). Final Technical Report (1994-97), project No. P – BZU/Environ. (29), Institute of pure and applied biology, Bahauddin Zakariya University, Multan.
- Jirka, A. M. and M.J. Carter (1975). Micro-Semi-Automated Analysis of surface and Wastewaters for Chemical Oxygen Demand. *Anal. Chem.*, 47:1397.
- Khan, A. R. and M. Akif (1994). *Technical Report*, PCSIR. Labs. Peshawar.
- Olsen, S. R. and L. E. Sommer (1982). Phosphorous total *In: Methods of soil analysis part 2. Chemical and Microbiological properties.* (Page A1. ed). Am.Soc. Agron. Inc. Madison, Wis. pp:403-430.
- Penaud, V., J.P. Delgenes., M.Torrijos, R. Moletta, B. Vanhoutte and P. Cans.(1997). Definition of optimal conditions for the hydrolysis and acidogenesis of a pharmaceutical microbial biomass. *In: Process biochemistry*, 32: 515-521.
- Yousaf, S. and Z.M. Khalid (2004). Microbiological means of detoxification of hazardous waste from an antibiotic producing pharmaceutical industry. *Int. J. Biol. Biotechnol.*, 1: 325-333.

(Accepted for publication 20 October 2004)